

ABSTRACT OF THE DISCLOSURE

5 The present invention relates to two ATP
diphosphohydrolases (ATPDase enzymes) isolated from
bovine aorta and pig pancreas, which enzymes have a
molecular weight for their catalytic unit of about 78
and 54 Kilodaltons, respectively. A first process for
obtaining a highly purified ATPDase is also an object of
the present invention. This process has been
successfully applied to the purification of both the
10 pancreatic and the aorta enzymes and is deemed to work
in the purification of any ATPDase. For both sources of
enzymes, the process allows the specific activity of the
enzyme to be increased by at least 10,000 fold when
compared to the activity retrieved in the crude cell
15 homogenates. The novel process involves an ion exchange
chromatography step, a separation on an affinity column,
followed by an electrophoresis under non-denaturing
conditions. The two enzymes purified by this process
(aortic and pancreatic) are glycosylated and, when
20 deglycosylated, have molecular weights shifted to about
56 and 35 Kdaltons, respectively. Partial amino acid
sequences have been obtained for each enzyme. The
partial sequences appear highly homologous with a human

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lymphoid cell activation antigen named CD39. An
antibody directed against the porcine pancreatic enzyme
cross-reacts with a protein present in endothelial cell
lines and in bovine aorta (78 KDa). The high degree of
5 homology of the pancreatic and aortic enzymes with CD39
and their cross-reactivity are indications that both
enzymes are related. The pancreatic enzyme completely
lacks the first 200 amino acids of CD39, which means the
ATPDase activity is comprised between residues 200 and
10 510 of CD39. Since this is the first time that a
sequence is assigned to ATPDases, a second new process
for producing ATPDases by recombinant technology can
also be used. Therefore a second new process for
producing an ATPDase using the CD39-encoding nucleic
15 acid or part or variant thereof is also described.

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